Melatonin on-line

Drijfhout, Willem Jan

Publication date:
1996

Chapter 6

6 Chronic effects of microdialysis probe implantation; a telemetry study

The present chapter describes the effects of implantation of microdialysis probes on temperature and activity rhythms of the rat, measured with a telemetry system. For comparison two widely used types of microdialysis probes were investigated, a trans cerebral probe, inserted into the pineal gland and a set of two I-shaped concentric probes, implanted bilateral into the striatum. Starting from 5 days before the operation until 8 days after the surgery, activity and temperature recordings were carried out continuously with the help of previously implanted transmitters. In separate experiments the effects of two different types of anaesthesia (chloral hydrate and Hypnorm®) were determined.

The results show that there is a pronounced effect of surgery on amplitude and rhythmicity of the temperature and activity patterns which is still detectable 6-7 days after operation. Few differences were noticed between the transversal probe and the I-shaped probes. Anaesthesia alone induced much smaller changes, most of which had disappeared within two or three days after the treatment. The duration of action of chloral hydrate is somewhat longer compared to Hypnorm®.

The conclusion is made that when microdialysis is used in behavioural experiments, the effects of the surgical procedure should be taken into account. If these effects are serious, the use of previously implanted guide cannulas might be necessary.

Data presented in this chapter are published in the following paper:

Drijfhout WJ, Kemper RHA, Meerlo P, Koolhaas JM, Grol CJ and Westerink BHC (1995) A telemetry study on the chronic effects of microdialysis probe implantation on the activity pattern and temperature rhythm of the rat *J. Neurosci. Meth.* **61**, 191-196.

6.1 Introduction

With increasing frequency microdialysis is used to analyse the neurotransmitter output during behavioural situations. The method has been used in several behavioural studies concerned with feeding, drinking and sexual activity.[71,121,270,410] An extensive review concerning this subject has been published recently.[409]

In microdialysis studies probes are often implanted one or two days before the behavioural recordings are carried out. However the effect of the surgery and anaesthesia on physiological and behavioural parameters of rats have received little attention so far.

Until recently, the techniques used to measure such physiological and behavioural parameters were mostly invasive and could therefore interfere with the parameters itself. The relatively new technique of telemetry has shown to be a very suitable technique for the undisturbed measurement of temperature, activity, heart rate and electrocardiograms.[60,164,369] This technique was applied to study the effect of a microdialysis probe implantation on the temperature and activity rhythm of rats.

Different probe designs result in different surgical procedures and might therefore give different results in such experiments. To examine those possible differences, two widely used probe designs were tested; a transversal probe, implanted in the pineal gland, requiring relatively large injuries during the operation and an I-shaped probe bilaterally implanted in the striatum.

To discriminate between effects of the surgery and the anaesthesia, also the effects of two different kinds of anaesthetics were examined. One was chloral hydrate, a hypnotic that is widely used in microdialysis experiments. The other was Hypnorm®, a commercially available mixture of fentanyl citrate (narcotic analgesic) and fluanisone (tranquillizedr). The advantage of this anaesthetic is its short duration of action, but on the other hand especially fentanyl, a mu-opiate receptor agonist, has been reported to induce various physiological changes.[21,261]

6.2 Experimental procedures

■ Animals and surgery

In contrast to other studies described in this thesis, the present experiments were carried out at the Department of Animal Physiology, Haren, University of Groningen. Being part of a larger experiment, a different strain of animals was used, namely male Tryon maze dull S3 rats of about 5 months of age (Department of Animal Physiology, University of Groningen). Other experimental conditions were similar to the ones described on page 58.

Two types of probes were tested, concentric (I-shaped) probes and trans pineal probes. The former were prepared as described by Santiago and Westerink.[308] The dialysis tube (i.d. = 0.22 mm; o.d. = 0.31 mm) was prepared from polyacrylonitrile/sodium methalyl sulfonate copolymer (AN 69, Hospal, Bologna, Italy). Probes were implanted bilateral. Coordinates of the implantation were : A/P + 0.7 mm and L/M 2.5 mm to bregma, V/D 6.0 mm to dura mater, according to the atlas of Paxinos and Watson.[246] The trans pineal probes were prepared (page 55) and implanted (page 59) as described previously.

■ Data collection

Body temperature and gross motor/movement activity were measured by means of radio telemetry. Transmitters (model TA11ETA-F40-L20 or TA10TA-F40, Data Sciences, St. Paul, Minnesota/MN, USA) were implanted intraperitoneally under ether anaesthesia at least three weeks before microdialysis probe implantation, so no possible negative side-effects of the ether anaesthesia will be expected at the time of experiments. Signals from the transmitters were picked up by a receiver board (model RA1010, Data Sciences) underneath the cages. Data were fed into a personal computer and processed with a specialized recording and analysis system (Dataquest IV, Data Sciences). Data were collected continuously at 10 minute intervals.

■ Treatment

In the first experiment two groups of rats were operated, one by implantation of two I-shaped probes in the striatum (n = 4), one by implantation of a transversal probe in the pineal gland (n = 6). Telemetric recordings were made from five days before the surgery until eight days after the surgery. Surgery was performed during the first hours of the light period.

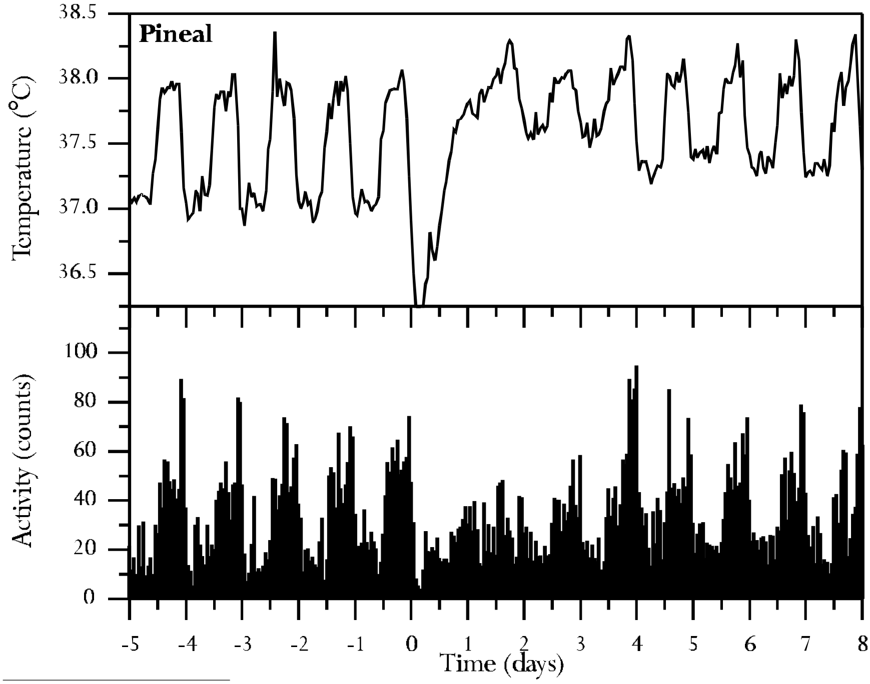

Figure 6.1 Telemetric temperature (upper panels) and activity (lower panels) recordings of rats that received trans pineal probe implantation (n = 6). Surgery was performed on day 0. Data were collected every 10 minutes and averaged to one hour samples. Lowest temperature measured was 33°C, shortly after the surgery.

In the second experiment two groups of rats were injected with anaesthetics. One group (n = 6) received a dose of 400 mg/kg i.p. chloral hydrate. The other group (n = 3) was injected with a commercially available combination of fentanyl citrate and fluanisone in a dose of 0.15 ml/100g i.p. (Hypnorm®). Telemetric recordings were made from four days before treatment until seven days after treatment. Anaesthetics were injected within the first hour after lights on.

■ Data analysis

Data collected at 10 minute intervals were averaged to one hour intervals. For each day the amplitude, as a marker of rhythmicity, of both temperature and activity was calculated by subtracting mean daytime values from mean night-time values. The resulting amplitude data were statistically evaluated. The effect of treatment on rhythmicity was tested by paired t-tests on data from subsequent days after treatment, compared to combined basal levels. Repeated measures ANOVA's were used to test for differences between both surgical procedures, both anaesthetics, and for differences between anaesthesia and surgery.

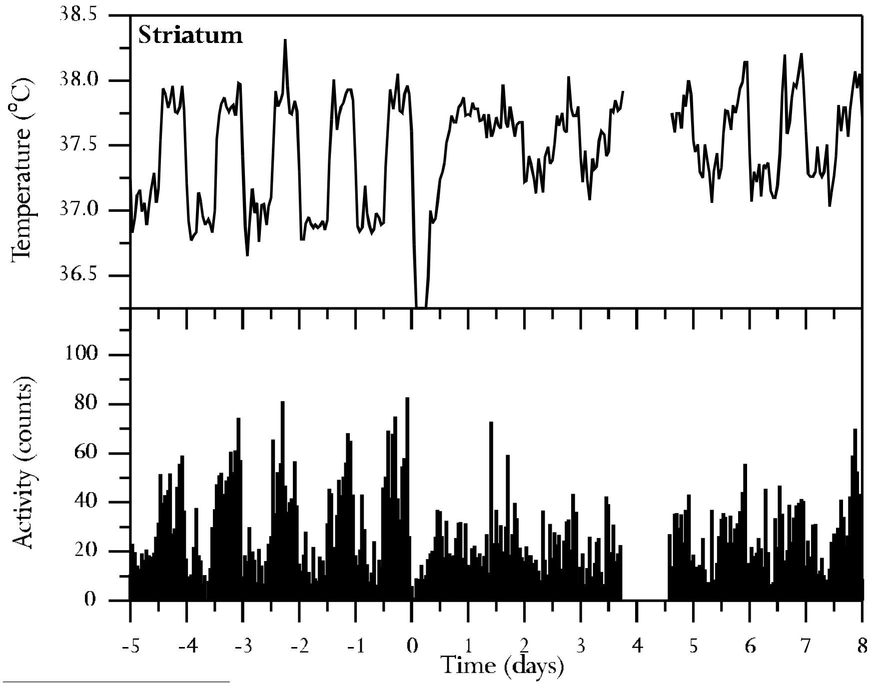

Figure 6.2 Telemetric temperature (upper panels) and activity (lower panels) recordings of rats that received bilateral striatal probe implantation (n = 4). Surgery was performed on day 0. Data were collected every 10 minutes and averaged to one hour samples. Lowest temperature measured was 33°C, shortly after the surgery. On day 4 some data are lost, due to a computer-failure during the experiment.

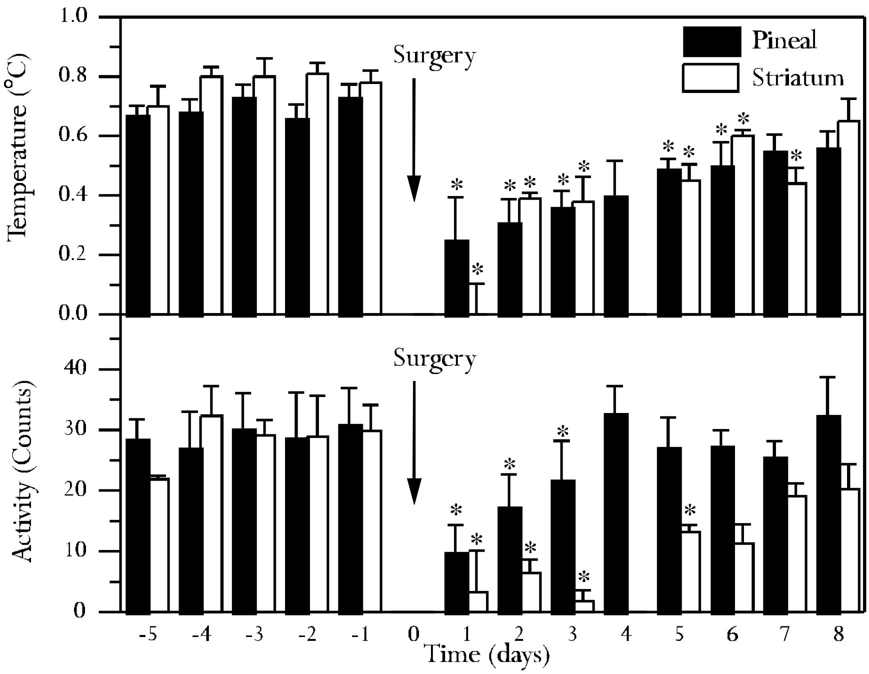

Figure 6.3 Amplitudes of temperature (upper panel) and activity (lower panel) rhythms before and after trans pineal (n= 6) and striatum (n = 4) probe implantation. Data are presented as mean ± S.E.M. Asterisks (*) indicate statistically significant differences with the mean basal values, as calculated by paired t-tests ($p < 0.05$).

6.3 Results

■ Temperature and activity rhythm of the rat

The temperature of all rats showed a clear circadian rhythm with high temperatures at the subjective night and low temperatures during subjective day. The amplitude was about 1°C. Because of variations between animals in the total activity and because of short-term fluctuations, the activity rhythms seemed less pronounced, but a clear circadian pattern of activity was visible with most activity occurring during the dark period. Since counts are only detected by the system when the transmitter moves relative to the receiver, activity must be considered as gross locomotor activity.

■ Effect of surgery on temperature and activity rhythm of the rat

During surgery the temperature of all animals dropped down to 33°C as could be seen from the recordings after the animals returned to their home cages. After both trans pineal (Fig. 6.1) and striatal (Fig. 6.2) probe implantation, levels were back to normal about four hours after surgery. However, the circadian rhythm of temperature was severely perturbed. Paired t-tests indicate that the amplitude was significantly decreased for 6-7 days after surgery (Fig. 6.3), mainly caused by a slight hyperthermia during the light period. This effect was essentially the same in both the implantation of the trans pineal (6 days) and the I-shaped probe (7 days).

Also the activity rhythms were severely perturbed both after trans pineal (Fig 6.1) and striatal (Fig. 6.2) probe implantation. Shortly after the surgery, activity counts returned to an average level, but with reduced amplitude. In the case of the I-shaped probe, paired t-tests revealed significant changes of normal rhythmicity until day 5 after the operation. Rats implanted with a trans pineal probe re-established their activity rhythm somewhat faster. Only significant changes were noted until day 3 after surgery. This difference in effect on activity between both operations was significant ($F(1,9)= 6{,}41$, $p = 0.032$).

■ Effect of anaesthesia on temperature and activity rhythm of the rat

Anaesthesia induced a short period of hypothermia which lasted for almost four hours. In the case of chloral hydrate (Fig. 6.4) the temperature dropped down to 33°C. The decrease in temperature during Hypnorm® anaesthesia (Fig. 6.5) was less and lasted only two hours. Despite this severe acute effect of the anaesthesia, circadian rhythmicity was not affected as strongly as with surgery. While circadian rhythmicity persisted, a decrease in amplitude of temperature was recorded, caused by elevated daytime levels, which was significant for 2 days in the case of chloral hydrate and did not reach the level of significance in the case of Hypnorm® (Fig. 6.6).

On the activity patterns, the effects of both anaesthetics were small and mainly restricted to a small decrease in amplitude. Because of the relative low number of subjects, especially for Hypnorm®, the level of significance was not reached. Neither is there any difference between the effects of the two anaesthetics.

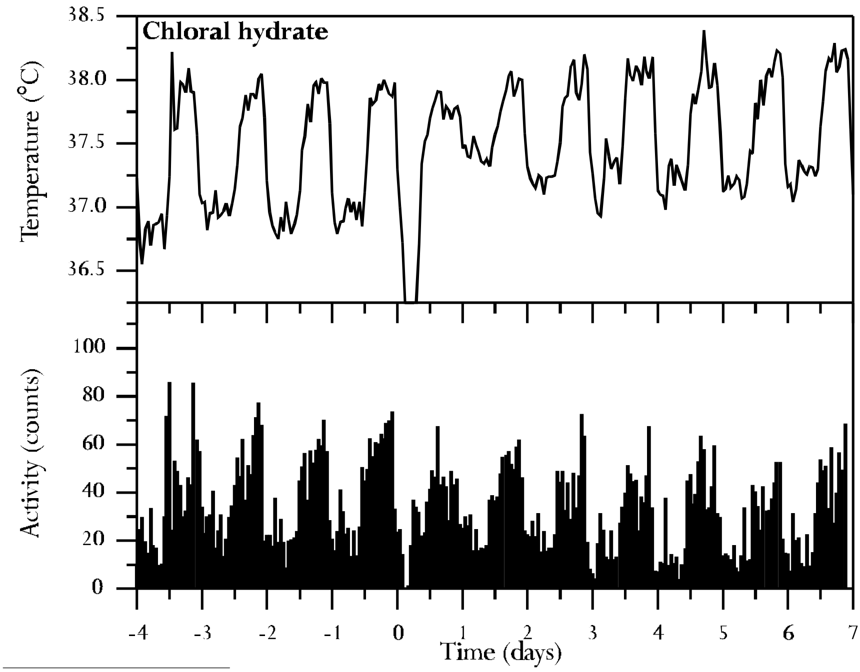

Figure 6.4 Telemetric temperature (upper panels) and activity (lower panels) recordings of rats that received chloral hydrate anaesthesia (n= 6). Anaesthetics were administered on day 0. Data were collected every 10 minutes and averaged to one hour samples.

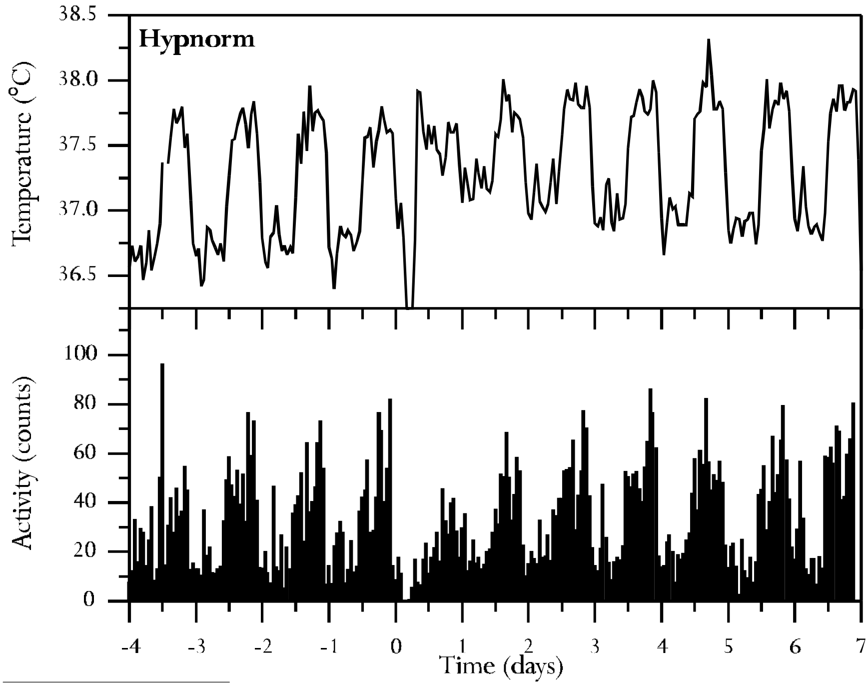

Figure 6.5 Telemetric temperature (upper panels) and activity (lower panels) recordings of rats that received Hypnorm® anaesthesia (n = 3). Anaesthetics were administered on day 0. Data were collected every 10 minutes and averaged to one hour samples.

When comparing the surgical procedure with anaesthesia, differences are apparent. Comparison of anaesthesia with pineal probe implantation shows a significant difference in effect on temperature ($F(1,10) = 79.16$, $p = 0.000$), while the difference in effect on activity rhythms is not significant. Comparing anaesthesia with striatal probe implantation, significant differences are measured in effect on both temperature ($F(1,8) = 78.87$, $p = 0.000$) and activity ($F(1,8) = 12.16$, $p = 0.008$) rhythms.

6.4 Discussion

The present data show that implantation of a microdialysis probe into the brain induces a long lasting disturbance of the rhythms of body temperature and activity of rats. The recorded circadian rhythms were affected both in amplitude and pattern. Normalisation of the studied rhythms took place within 7 days after implantation of the probe. In contrast to what was expected, no differences between two basically different probe designs were seen with respect to temperature. Apparently the bigger injury caused by implantation of the transversal probe, is not reflected on temperature. The activity rhythm however is more severely disrupted by the striatum probe than by the transversal

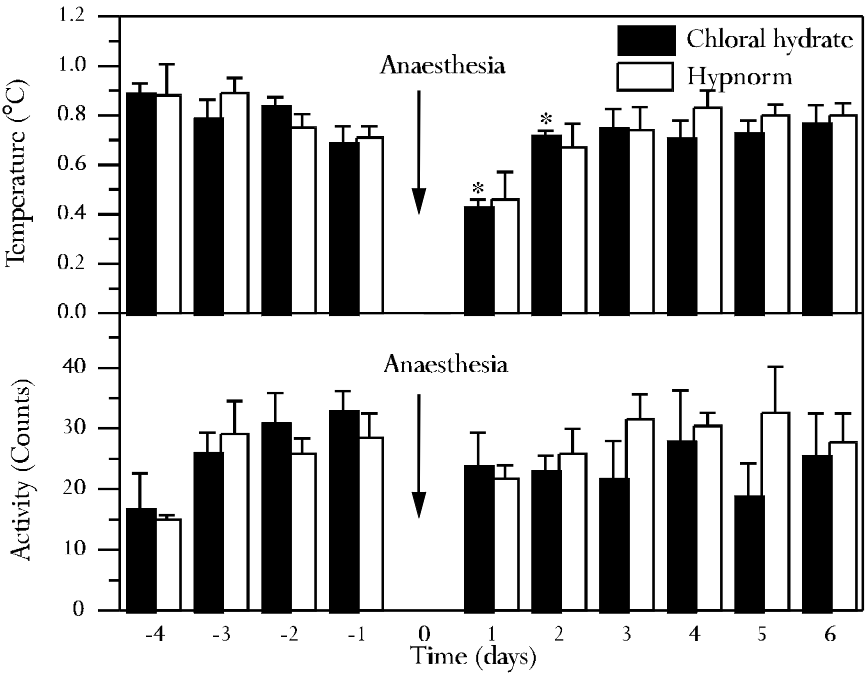

Figure 6.6 Amplitudes of temperature (upper panel) and activity (lower panel) rhythms before and after anaesthesia with chloral hydrate (n = 6) and Hypnorm® (n = 3). Data are presented as mean ± S.E.M. Asterisks (*) indicate statistically significant differences with the mean basal values, as calculated by paired t-tests ($p < 0.05$).

pineal probe. This could be due to brain damage caused by the striatum probe, an effect that will vary with the position of the probe in the brain.

Furthermore the disturbing effects seen, can be attributed mainly to the surgical procedure, since anaesthesia by itself does have much smaller, mostly acute, effects. This is in accordance with other studies in which anaesthetics applied either locally in the suprachiasmatic nucleus (SCN),[316] or systemically,[63] did not affect circadian rhythmicity. The major difference between the two anaesthetics used is the somewhat stronger temperature drop and longer duration of action during chloral hydrate anaesthesia. This difference is also reflected in a significant change in temperature amplitude the first two days after chloral hydrate treatment. Chloral hydrate as an hypnotic agent is known to have a long lasting effect. Also, although widely used in microdialysis experiments, it is reported that chloral hydrate is slightly more stressful than Hypnorm®.[96] Despite these effects, differences in temperature and activity are levelled off shortly after the anaesthesia. Therefore, as far as rhythmicity is concerned, the choice of the anaesthetic seems not to be a critical one.

The temperature drop to about 33 °C in both anaesthesia and surgery is substantial. Temperature regulation during anaesthesia and surgery with a heating pad, possibly based on continuous measurement of rectal temperature can therefore be advisable. However, improvement of the temperature and activity rhythms during subsequent days after surgery will not be improved, since anaesthesia by itself, which induces large hypothermia, does not show long term effects.

The increase of the daytime temperature for 6-7 days after the surgery could be explained by a slight hyperthermia caused by wound healing. However, since anaesthesia by itself shows a similar effect, it could be that this is a general effect of anaesthesia. Preliminary results from similar experiments in which heart rate was measured show an increase in frequency after both surgery and anaesthesia, thus confirming more general effects of anaesthesia. Control experiments in which saline was injected (data not shown) exclude any influence of the injection procedure.

Although traumatic (calcium-independent) neurotransmitter release has been reported to occur during the first hours after implantation of a probe, in general neurotransmitter release displays physiological properties 8-24 h after surgery.[308] It is evident from the present study that various homeostatic processes in the body are not yet recovered at that stage. Its implication for any experiments performed during the first days after the surgery remains speculative, but possible influences on various experiments must not be excluded and need further research.

The present results do not mean that relevant neurochemical or behavioural experiments can not be carried out during the first days after implantation of a microdialysis probe. Feeding induced increase in dopamine release in the nucleus accumbens is a response that is already detectable 24 h after implantation of the probe and this response remained constant for at least 6 days after implantation.[410] Also dopamine increases in the nucleus accumbens induced by sexual behaviour seem not to be affected by the surgical procedure.[71] Furthermore, in this thesis it is shown that the circadian rhythm in the release of noradrenaline and melatonin from the pineal gland is not affected by the implantation of the probe. Twentyfour hours after implantation of the probe a characteristic and very robust circadian rhythm was detected that was strongly related to the day/night rhythm (chapter 7). This rhythm was comparable to rhythms measured with other techniques and the same during subsequent days, indicating that this system is functioning well, even shortly after the surgery.

In conclusion, when microdialysis is used in subtle behavioural observations, side effects related to the surgery are to be expected. When behavioural experiments are carried out in animals equipped with an acutely implanted microdialysis probe, it should be taken into account that hormonal and autonomic functions can be disturbed for at least one week after implantation. Moreover implantation in more vulnerable regions such as the midbrain and brainstem might have even stronger effects on these parameters. In case these interferences occur, they might be overcome by using probes equipped with a guide cannula. The present data show that it is advisable to implant such guide cannulas at least one week before the experiments are carried out.

The most important conclusion must be that effects of the surgical procedure involved in implanting microdialysis probes can be serious. Therefore it should be tested in the various applications of this technique.